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Published in:
Carbohydrate Polymers

DOI:
10.1016/S0144-8617(99)00123-X

Publication date:
2000

ELSEVIER

Carbohydrate Polymers 42 (2000) 33–43

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Synthesis and reduction of 2-nitroalkyl polysaccharide ethers

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Received 22 March 1999; received in revised form 7 June 1999; accepted 25 June 1999

Abstract

Several 2-nitroalkyl polysaccharide ethers (from pullulan (**1**), guar (**2**), agarose (**3**), inulin (**4**), cellulose (**5**), Na-α-polyglucuronate (**6**) and hydroxyethyl cellulose (**7**)) were synthesized by reaction with 2-nitro-1-alkenes (2-nitro-1-propene and 2-nitro-1-butene) formed in situ from 2-nitroalkyl acetates. Moderate to high efficiencies are obtained in concentrated aqueous solution/suspension for addition to **1**–**4** and **7**. Analysis of this new class of polysaccharide derivatives with the aid of labeled 2-nitropropyl-2-^{13}C pullulan revealed that the nitrogroup is a mixture of the nitroalkane and nitronic acid tautomers. Grafting of nitroalkenes is observed and, to a lesser extent, additional reactions of the nitro group (formation of carbonyl, oxime and allyl groups) take place.

Reduction of 2-nitroalkyl polysaccharide ethers with $Na_2S_2O_4$ or $Na_2S_2O_4$/$NaBH_4$ leads to complex polysaccharide ethers. The products obtained are most likely mixtures of starting material, nitroso compounds, hydroxylamines, hydroxypropyl ethers and sulfamates.

Keywords: Polysaccharides; Modification; Nitroalkylation; Reduction

1. Introduction

In the literature, various claims are made and attempts described to synthesize polysaccharides with primary or secondary amino functionalities (Cimecioglu, Ball, Kaoplan & Huang, 1994; Daly & Munir, 1984; Hebeish, Waly, Higazy & Abdel-Mahdy, 1994; Kerr & Neukom, 1952; Verraest, Silva, Peters & van Bekkum, 1996; Verraest, Zitha-Bovens & van Bekkum, 1998; Wolfrom, Taha & Horton, 1963). However, none of the routes to these are suitable for large scale synthesis. Modified polysaccharides with primary or secondary amino groups are expected to have interesting properties for a number of reasons: amino-polysaccharides have potential for the immobilization of enzymes (Guiseley & Yalpani, 1987; Zaborsky, 1974), as ion-exchange resins (Bullock & Guthrie, 1965), for drug targeting (Fuchs, 1993) and for complexation of heavy metals in polluted water (Nakamura, Amano, Saegua & Sato, 1992). The amino functionality, which can easily be converted into other functionalities, makes these compounds excellent precursors for a wide variety of other secondary polysaccharide derivatives. The rheology of the aminopolysaccharide derivatives is pH-dependent, owing to the basicity of the amino functionality.

Although nature has provided us with the primary amino-polysaccharide chitosan, the solubility of chitosan in water or alcohols is low and modifications of chitosan are usually performed in “exotic” solvents. Naturally, this lowers the attractiveness for modifications on an industrial scale.

Our attention was drawn by the work of Gruber and Greber (1991). They developed a novel route for preparation of 2-aminoalkyl sucrose ethers by Michael additions of nitroalkenes (or precursors thereof) to sucrose and subsequent reduction of the nitroalkyl ether to the corresponding amine by catalytic hydrogenation. We decided to investigate the potential of this method for the synthesis of 2-aminoalkyl polysaccharide ethers in the realization that another reducing agent would have to be used because it is well known that heterogeneous catalytic conversions of polymers are often complicated and low conversion rates are usually obtained (Scheme 1 [Proposed route for the synthesis of 2-aminoalkyl polysaccharide ethers (pullulan, C-2 substitution is taken as an example)]) (Verraest, Peters & van Bekkum, 1998; Verraest et al., 1998).

Not only are the 2-nitroalkyl polysaccharide ethers

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0144-8617/00/$ - see front matter
PII: S0144-8617(99)00123-X

potential precursors for aminoalkyl polysaccharides, they are also interesting compounds themselves. Interesting rheological behavior is expected owing to the polar, slightly acidic and hydrophobic nitroalkyl ether substituent. Furthermore, the nitro functionality can easily be transformed into other functionalities (Rosini & Ballini, 1988; Seebach, Colvin, Lehr & Weller, 1979; Tamura, Kamimura & Ono, 1991), e.g. keto functionalities (modified Nef reactions see Kornblum & Wade, 1973; Olah, Arvanghi, Vankar & Prakash, 1980).

In order to facilitate the analysis of the products obtained, a number of model compounds as well as compounds based on ^{13}C-enriched starting material were prepared.

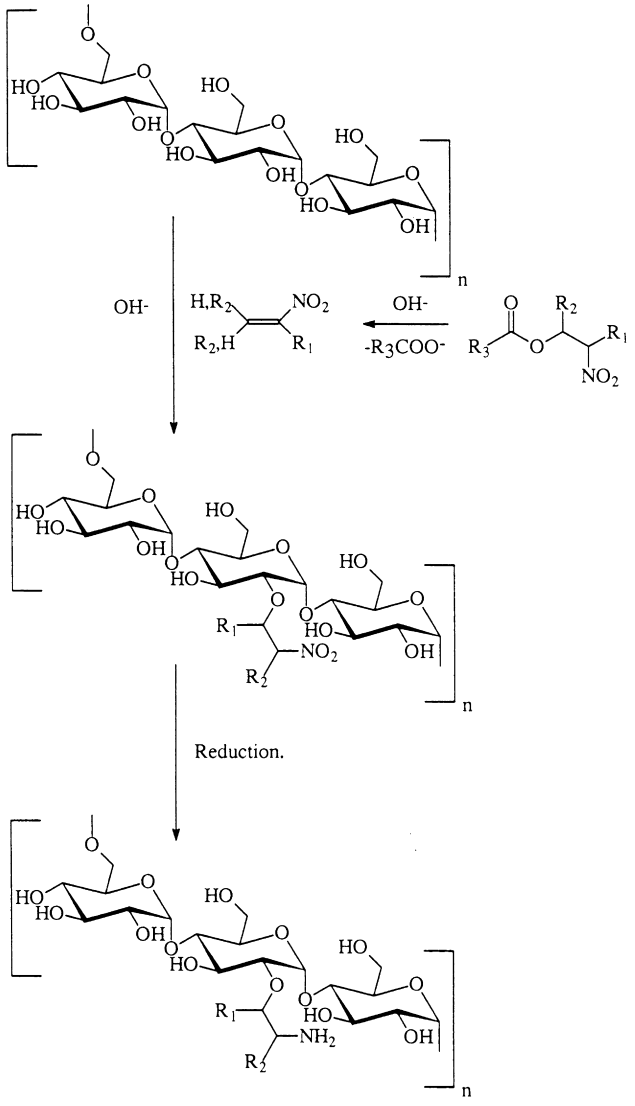
2. Experimental

2.1. General methods and starting materials

2-Nitropropanol and 2-nitrobutanol were synthesized according to a procedure from the literature (Feuer & Miller, 1961). 2-Nitropropyl acetate, 2-nitropropyl acetate-2-^{13}C and 2-nitrobutyl acetate were prepared according to slightly modified literature procedures (Padeken, Schick & Segnitz, 1971). The synthesis of 2-nitropropyl acetate is given as a general example.

Synthesis of 2-nitropropyl acetate. Acetic anhydride (3850 g, 37.7 mol) and 2-nitropropanol (3600 g, 34.28 mol) were divided into 20 portions of about 300 ml. A few drops of concentrated H_2SO_4 were added to each portion. After 15 min the mixtures were combined and stirred for 16 h. The reaction mixture was poured into Et_2O (15 l) and neutralized with saturated $NaHCO_3$ solution (15 l). After separation of the organic layer, the $NaHCO_3$ solution was washed with Et_2O (5 l) and the combined ether layers were dried over Na_2SO_4. After filtration and rotary evaporation of the solvent, the residue was distilled under reduced pressure (0.1 mm Hg, 75°C) and 4373 g of 2-nitropropyl acetate (29.75 mol, 87%) was obtained. NMR ($CDCl_3$, in ppm): ^1H, δ 1.55 (d, 3H, CH_3), δ 2.05 (s, 3H, $O=CCH_3$), 4.41 (d, 2H, CH_2), 4.77 (m, 1H, CH). ^{13}C, δ 15.37 (CH_3), 20.26 (CH_3), 64.16 (CH_2), 80.84 (CH), 170.16 (C=O).

Pullulan (dry substance content 89.89%) and guar (dry substance content 89.16%) were available within AVEBE (Foxhol, The Netherlands). Agarose (dry substance content 92.68%) and cellulose (dry substance content 95.83%) were purchased from Sigma. Hydroxyethylcellulose (dry substance content 89.91%) was a gift from Aqualon (Zwijndrecht, The Netherlands). Inulin (dry substance content 96.49%) was a gift from Sensus (Roosendaal, The Netherlands). Sodium α-polyglucuronate was synthesized according to a literature procedure (De Nooy, Besemer & van Bekkum, 1994). Nitroethane (96%) was purchased from Aldrich. Nitroethane-1-^{13}C was obtained from Campro Scientific (Veenendaal, The Netherlands). Formaldehyde



Scheme 1.

(37% solution), acetic acid anhydride and 1-nitropropane (95%) were purchased from Aldrich. NaOH pellets and $NaBH_4$ were purchased from Merck. $Na_2S_2O_4$ was obtained from Fluka.

^{13}C-NMR spectra were recorded on a Varian 500 MHz spectrometer. FT-IR spectra were recorded on a Biorad FTS 135 spectrometer. The pH-stat, a Titration Controller 1200 equipped with a Titronic T110 burette, was purchased from Schott Geräte. Spectra/Por membranes (MWCO: 2000 and MWCO: 500, for dialysis) were obtained from Spectrum Medical Industries. Dry substance contents of the polysaccharides and modified polysaccharides were determined by drying for 2 h at $T = 130$°C. Nitrogen analyses were performed at the Analytical Department of AVEBE (Foxhol, The Netherlands). Sulfur analyses were performed at the micro-analytical department of the University of Groningen.

Table 1
Synthesis of 2-nitropropyl polysaccharide ethers

Polysaccharide	Amount	2-npa[a]	NaOH	H_2O	Yield	%N
Pullulan (**1**a)	10.01 g (55.5 mmol)[b]	2.40 g (16.3 mmol)	1.35 g (33.8 mmol)	20 ml	8.39 g	2.21
Pullulan (^{13}C-**1**a)	1.00 g (5.55 mmol)	0.206 g (1.4 mmol)[c]	0.11 g (2.75 mmol)	2.5 ml	0.85 g	n.d.
Guar (**2**a)	10.00 g (55.0 mmol)	2.40 g (16.3 mmol)	1.33 g (33.3 mmol)	20 ml	9.46 g	2.32
Agarose (**3**a)	10.00 g (60.6 mmol)	2.40 g (16.3 mmol)	1.35 g (33.7 mmol)	20 ml	10.07 g	2.00
Agarose (**3**b)	13.86 g (84.1 mmol)	2.30 g (15.6 mmol)	1.35 g (33.8 mmol)	145 ml	12.90 g	0.83
Agarose (**3**c)	5.51 g (35.5 mmol)	1.26 g (8.60 mmol)	0.67 g (16.8 mmol)	140 ml[d]	4.96 g	0.48
Inulin (**4**a)	25.00 g (148 mmol)	6.00 g (40.8 mmol)	3.35 g (84.0 mmol)	25 ml	10.47 g	1.40
Cellulose (**5**a)	9.98 g (59.0 mmol)	2.42 g (16.4 mmol)	1.36 g (34.0 mmol)	20 ml	5.52 g	0.27
α-polyglucuronate (**6**a)	10.02 g (50.0 mmol)	6.17 g (42.0 mmol)	4.00 g (100 mmol)	25 ml	8.00 g	1.71
HE-cellulose (**7**)	10.00 g (29.6 mmol)	2.40 g (16.3 mmol)	1.37 g (34.3 mmol)	20 ml	9.17 g	1.09

[a] 2-npa = 2-nitropropyl acetate.
[b] Amount of glucose monomers.
[c] 50% enriched with 2-nitropropyl-2-^{13}C acetate.
[d] 2-propanol/H_2O 13:1.

2.2. Synthesis of 2-nitroalkyl polysaccharide ethers (and model compounds)

Synthesis of 2*-nitroalkyl polysacharide ethers, general example.* 2-Nitroalkyl acetate was added dropwise to a solution/suspension of the polysaccharide in H_2O containing 2 equivalents of NaOH. After 3 h stirring additional H_2O was added and the solution was neutralized with 4.0 N HCl. After dialysis (MWCO: 2000) and freeze-drying (**1**–**3**, **7**), or precipitation in organic solvents (**4**–**6**, EtOH, MeOH), the corresponding 2-nitroalkyl polysaccharide ethers were isolated (see Tables 1 and 2).

Synthesis of 1*-methoxy*-2*-nitropropane.* 2-Nitropropyl acetate (9.13 g, 62.1 mmol) was added dropwise at $T = 0$°C to a solution of MeOH (160 ml) and NaOMe (30% solution in MeOH, 18.5 ml, 240 mmol). After stirring for 24 h and rotary evaporation of the solvent, H_2O (40 ml) was added. After addition of acetic acid (16 ml) at $T = 0$°C the water layer was extracted with Et_2O (3×75 ml). The combined Et_2O layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was distilled under reduced pressure (15 mm Hg, 80°C) and 1-methoxy-2-nitropropane (2.68 g, 22.5 mmol, 36%) was isolated. NMR (200 MHz, $CDCl_3$, in ppm): ^{1}H, δ 1.50 (d, 3H, CH_3), 3.35 (s, 3H, OCH_3), 3.60 (dd, 1H, CH_2, H_a), 3.80 (t, 1H,

Table 2
Synthesis of 2-nitrobutyl polysaccharide ethers

Polysaccharide	Amount	2-nba[a]	NaOH	H_2O	Yield	%N
Pullulan (**1**b)	10.04 g (55.7 mmol)	2.68 g (16.6 mmol)	1.34 g (33.5 mmol)	20 ml	8.83 g	1.71
Guar (**2**b)	10.13 g (55.8 mmol)	2.61 g (16.2 mmol)	1.34 g (33.5 mmol)	20 ml	9.04 g	2.22
Agarose (**3**b)	10.07 g (61.0 mmol)	2.69 g (16.7 mmol)	1.34 g (33.5 mmol)	20 ml	9.98 g	1.47
Inulin (**4**b)	24.87 g (148 mmol)	6.30 g (39.1 mmol)	3.36 g (84.0 mmol)	25 ml	15.78 g	1.63
Cellulose (**5**b)	10.20 g (60.3 mmol)	2.70 g (16.8 mmol)	1.35 g (33.8 mmol)	20 ml	8.88 g	0.46
α-polyglucuronate (**6**b)	10.00 g (50.0 mmol)	10.00 g (62.1 mmol)	5.02 g (126 mmol)	25 ml	9.99 g	1.32

[a] 2-nba = 2-nitrobutyl acetate.

Table 3
Reduction of 2-nitropropyl polysaccharide ethers ($Na_2S_2O_4$)

2-nitropropyl polysaccharide ether	Amount	$Na_2S_2O_4$	H_2O	Yield	%N
Pullulan (**1**a)	2.32 g	4.69 g (22.3 mmol)	50 ml	2.06 g	1.82
Pullulan (^{13}C-**1**a)	0.50 g	0.90 g (4.28 mmol)	10 ml	0.45 g	n.d.
Guar (**2**a)	2.57 g	5.02 g (23.9 mmol)	50 ml	2.39 g	2.00
Agarose (**3**a)	2.46 g	4.65 g (22.1 mmol)	50 ml	1.58 g	1.78
Inulin (**4**a)	2.35 g	4.35 g (20.7 mmol)	50 ml	1.20 g	0.54
α-polyglucuronate (**6**a)	1.96 g	1.48 g (7.1 mmol)	20 ml	1.48 g	1.58, %S = 0.84%
HE-cellulose (**7**)	2.50 g	4.48 g (21.3 mmol)	50 ml	2.09 g	0.87

CH_2, H_b), 4.75 (m, 1H, CH). ^{13}C, δ 15.27 (CH_3), 58.94 (OCH_3), 73.37 (CH_2), 82.05 ($CHNO_2$).

Synthesis of 1-*methoxy*-2-*nitrobutane.* 2-Nitrobutyl acetate (7.50 g, 46.6 mmol) was added dropwise at $T = 0$°C to a solution of MeOH (120 ml) and NaOMe (14 ml, 30% solution in MeOH, 182 mmol). After stirring for 20 h and rotary evaporation of the solvent, H_2O (30 ml) was added. After addition of acetic acid (12 ml) at $T = 0$°C the water layer was extracted with Et_2O (3 × 75 ml). The combined Et_2O layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was distilled under reduced pressure (12 mm Hg, 75°C) and 1-methoxy-2-nitrobutane (2.32 g, 17.5 mmol, 37%) was isolated. NMR (200 MHz, $CDCl_3$, in ppm): ^{1}H, δ 0.96 (t, 3H, CH_3), 1.82 (m, 2H, CH_2), 3.33 (s, 3H, OCH_3), 3.58 (dd, 1H, CH_2, H_a), 3.82 (t, 1H, CH_2, H_b), 4.60 (m, 1H, $CHNO_2$). ^{13}C, δ 9.79 (CH_3), 23.22 (CH_2), 58.89 (OCH_3), 72.35 (CH_2), 88.75 ($CHNO_2$).

Synthesis of 1-*methoxy*-2-*methyl*-2,4-*dinitropentane.* - 1-Methoxy-2-nitropropane (1.01 g, 8.5 mmol) was added at $T = 0$°C to a solution of NaOH (0.32 g, 8.0 mmol) and NaAc (0.70 g, 8.5 mmol) in MeOH/H_2O (3:2, 5 ml). After stirring for 1 h at T_k the solution was cooled to 0°C and 2-nitropropyl acetate (1.25 g, 8.5 mmol) was added dropwise. After stirring for 20 h and rotary evaporation of the solvent H_2O (10 ml) was added. The water layer was extracted with Et_2O (3 × 15 ml) and the combined Et_2O layers were dried over Na_2SO_4. After filtration and evaporation of the solvent, the residue was distilled under reduced pressure (Kugelröhr, 0.1 mm Hg, 100°C) and both diastereoisomers of 1-methoxy-2-methyl-2,4-dinitropentane (0.18 g, 0.87 mmol, 10% (not optimalized)) were isolated. NMR (200 MHz, $CDCl_3$, in ppm): ^{1}H, δ 1.49 (s, 3H, CH_3) 1.56 (d, 3H, CH_3), 2.43 (t, 1H, CH_2, H_a), 2.82 (m, 1H, CH_2, H_b) 3.16 (s, 3H, OCH_3), 3.27 (s, 3H, OCH_3), 3.52 (t, 1H, OCH_2, H_a), 3.81 (m, 1H, OCH_2, H_b), 4.78 (m, 1H, $CHNO_2$). ^{13}C, δ 19.00, 21.28 (CH_3 (q)), 21.91, 22.87 (CH_3 (t)), 39.00, 39.78 (CH_2), 58.34, 59.03 (OCH_3), 75.30, (OCH_2), 79.55, 79.66 ($CHNO_2$), 88.31, 88.79 (CNO_2).

2.3. Reduction of 2-nitroalkyl polysaccharide ethers (and model compounds)

Reduction of 2-*nitroalkyl polysaccharide ethers*, *general example.* $Na_2S_2O_4$ was added in portions to a solution of a 2-nitroalkyl polysaccharide in H_2O. The pH was regulated with a pH-stat (pH = 7) by adding portions of 1 M NaOH. After 16 h the solution was dialyzed and freeze-dried.

A slightly modified procedure was followed for reductions with $Na_2S_2O_4$/$NaBH_4$. After reaction the solution was acidified with 4 N HCl to pH = 3. MeOH was added and after concentration, the solution was dialyzed and freeze-dried (see Tables 3–5).

Reduction of 1-*methoxy*-2-*nitropropane with* $Na_2S_2O_4$ (*NMR tube reaction*). 1-Methoxy-2-nitropropane (0.075 g, 0.62 mmol), Na_2CO_3 (0.16 g, 1.51 mmol) and $Na_2S_2O_4$ (0.50 g, 2.38 mmol) were dissolved in 2 ml D_2O and the reaction was followed with NMR. NMR (after 24 h, 200 Mhz, D_2O, in ppm): (major product) ^{1}H δ 1.03 (3H,

Table 4
Reduction of 2-nitropropyl polysaccharide ethers ($Na_2S_2O_4$/$NaBH_4$)

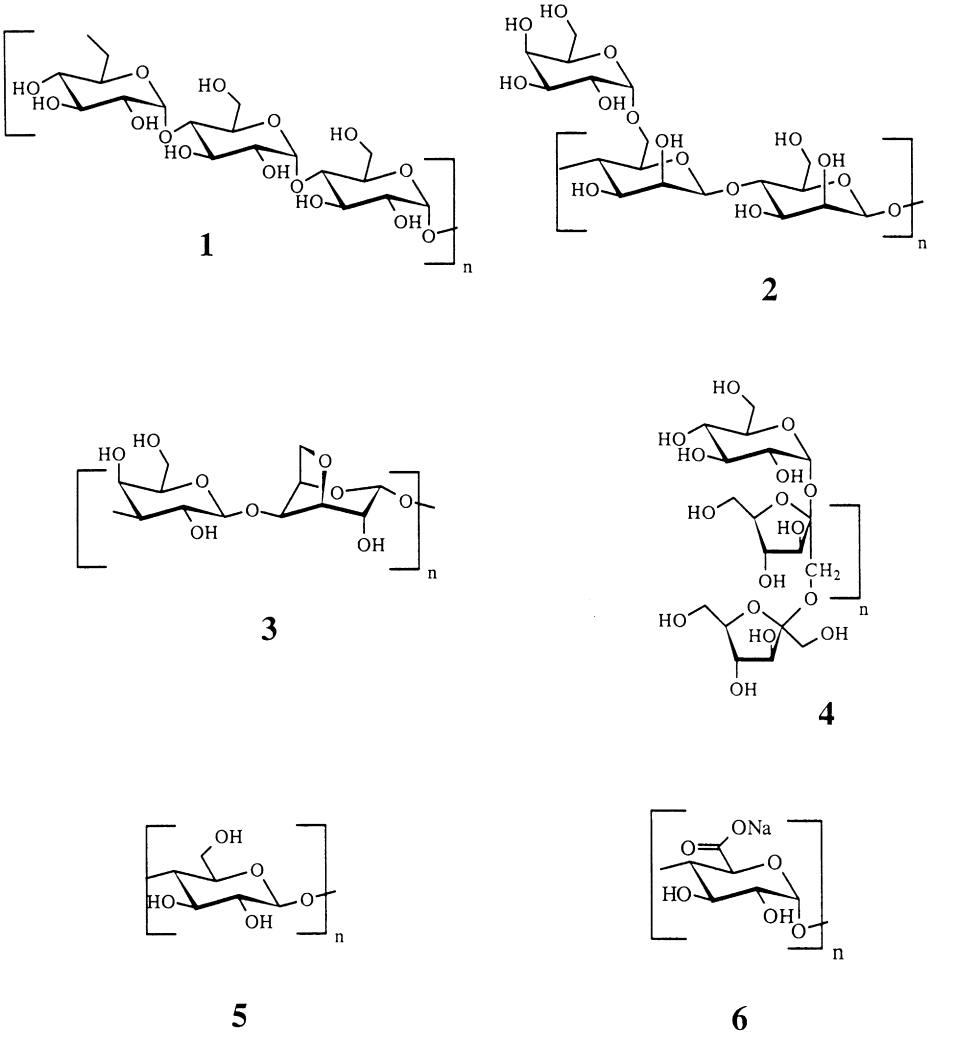
2-nitropropyl polysaccharide ether	Amount	$Na_2S_2O_4$	$NaBH_4$	H_2O	Yield	%N
Pullulan (**1**a)	2.48 g	4.50 g (21.4 mmol)	0.35 g (9.3 mmol)	50 ml	2.07 g	1.56
Pullulan (^{13}C-**1**a)	0.19 g	0.36 g (1.71 mmol)	0.025 g (0.67 mmol)	10 ml	0.17 g	n.d.
Guar (**2**a)	2.52 g	4.50 g (21.4 mmol)	0.35 g (9.3 mmol)	200 ml	1.96 g	2.47
Agarose (**3**a)	2.53 g	4.50 g (21.4 mmol)	0.35 g (9.3 mmol)	50 ml	1.99 g	1.66
HE-cellulose (**7**)	2.51 g	4.50 g (21.4 mmol)	0.35 g (9.3 mmol)	100 ml	2.25 g	0.76

Table 5
Reduction of 2-nitrobutyl polysaccharide ethers ($Na_2S_2O_4$)

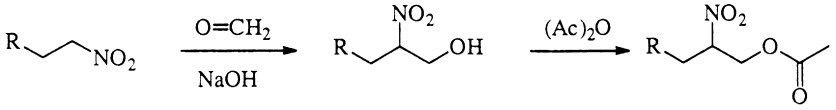
2-nitropropyl polysaccharide ether	Amount	$Na_2S_2O_4$	H_2O	Yield	%N
Pullulan (**1**b)	2.16 g	4.83 g (23.0 mmol)	50 ml	1.86 g	1.54
Guar (**2**b)	2.33 g	4.69 g (22.0 mmol)	50 ml	1.86 g	1.77
Agarose (**3**b)	2.47 g	4.30 g (20.5 mmol)	50 ml	2.36 g	1.59
Inulin (**4**b)	2.35 g	4.63 g (22.0 mmol)	50 ml	1.53 g	0.38
α-polyglucuronate (**6**b)	1.94 g	1.53 g (7.3 mmol)	20 ml	1.87 g	1.10

d, H_3), 3.30 (m, 5H, OC**H**$_3$ + OC**H**$_2$). ^{13}C δ. 17.08 (**C**H_3), 46.41 (**C**H), 58.14 (O**C**H_3), 75.45 (O**C**H_2), (minor product) ^{1}H δ 1.14 (3H, d, **C**H_3), 3.30 (m, 5H, O**C**H_3 + O**C**H_2). ^{13}C δ 14.22 (**C**H_3), 46.41 (**C**H), 58.14 (O**C**H_3), 72.69 (O**C**H_2).

Reduction of 1-*methoxy*-2-*nitrobutane with* $Na_2S_2O_4$ (*NMR tube reaction*). 1-Methoxy-2-nitropropane (0.082 g, 0.62 mmol), Na_2CO_3 (0.16 g, 1.51 mmol) and $Na_2S_2O_4$ (0.50 g, 2.38 mmol) were dissolved in 2 ml D_2O and the reaction was followed with NMR. NMR (after 24 h, 200 Mhz, D_2O, in ppm): (major product) ^{1}H δ 0.77 (t, 3H, C**H**$_3$), 1.42 (m, 2H, C**H**$_2$), 3.30 (m, 5H, OC**H**$_3$ + OC**H**$_2$). ^{13}C δ. 9.29 (**C**H_3), 24.16 (**C**H_2), 54.13 (O**C**H), 58.25 (O**C**H_3) 73.39 (O**C**H_2), (minor product) ^{1}H δ 0.84 (d, 3H, **C**H_3), 1.42 (m, 2H, CH_2), 3.30 (m, 5H, O**C**H_3 + O**C**H_2). ^{13}C δ 8.99 (**C**H_3), 21.90 (**C**H_2), 51.85 (**C**H), 58.56 (O**C**H_3), 70.89 (O**C**H_2).



Scheme 2.



Scheme 3.

3. Results and discussion

3.1. Synthesis of 2-nitroalkyl polysaccharide ethers

The polysaccharides pullulan (**1**), guar (**2**), agarose (**3**), inulin (**4**), cellulose (**5**) and sodium α-polyglucuronate (**6**, see Scheme 2 (Structure of pullulan (**1**), guar (**2**), agarose (**3**), inulin (**4**), cellulose (**5**) and sodium α-polyglucuronate (**6**).)) were chosen to investigate the scope of the Michael addition of nitroalkenes.

Previous experiments with granular potato starch showed high efficiencies for Michael additions to 2-nitro-1-alkenes formed in situ from β-nitro-acyloxy alkanes in concentrated aqueous systems (Heeres, van Doren, Gotlieb, Bleeker & Kellogg, 1997, 1998a,b). Although not commercially available, the reagents/precursors 2-nitropropyl and 2-nitrobutyl acetate can be synthesized in high yields from cheap starting materials (Scheme 3 [Synthesis of 2-nitropropyl- (R = H) and 2-nitrobutyl acetate (R = Me)]), and therefore, the production of 2-nitroalkyl ethers of polysaccharides could well become economically feasible.

The Michael additions of compounds **1**–**6** to 2-nitro-1-alkenes, formed in situ from the 2-nitroalkyl acetates, were performed in concentrated homogeneous (pullulan, inulin, sodium α-polyglucuronate) or heterogeneous (agarose, guar, cellulose) water systems. Two molar equivalents of NaOH per mole of 2-nitroalkyl acetate were added to maintain alkaline conditions. All reactions were performed at room temperature and the modified polysaccharides were purified by dialysis or precipitation in organic solvents. The results for the nitroalkylation of polysaccharides **1**–**6** are shown in Table 6.

High reaction efficiencies were obtained for the synthesis of 2-nitropropyl pullulan, -guar and -agarose. The reaction of **1**–**3** with 2-nitrobutyl actetate proceeded less efficiently, but still satisfactorily.

The efficiency of the nitroalkylation of inulin (**4**) could not be determined properly because during the work up procedure (precipitation in organic solvents or dialysis against MWCO 500) material was lost. Most likely, the low molecular weight inulin suffers from alkaline depolymerization during etherification. The β-(2 → 1)-linkage between the fructose units of inuline is rather labile and peeling off the polysaccharide chains from the thus formed reducing end results in the formation of lactates and glucosaccharinates (Whistler and BeMiller). An experiment in which the inulin was dialyzed before the nitroalkylation reaction suffered from the same loss of material during the work-up procedure. This supports the abovementioned hypothesis.

Nitroalkylation of the highly crystalline cellulose (**5**) proceeded with low efficiency. Higher efficiencies were obtained for hydroxyethyl (HE) cellulose (**7**, ms = 3.23). Maximization of the accessibility of cellulose by

Table 6
Nitroalkylation of polysaccharides **1**–**6**

Polysaccharide	2-nitropropyl acetate[a]			2-nitrobutyl acetate[a]		
	ms_{max}	ms	eff[b]	ms_{max}	ms	eff[b]
Pullulan (**1**)[c]	0.29	0.25	> 0.86	0.30	0.18	> 0.61
Guar (**2**)[c]	0.30	0.20	> 0.67	0.30	0.19	> 0.62
Agarose (**3**)	0.27	0.25	0.93	0.27	0.18	0.67
Inulin (**4**)	0.28	0.18	0.66[d,e]	0.30	0.30	1.00[d,e]
Cellulose (**5**)	0.28	0.03	0.11	0.28	0.05	0.18
Sodium α-polyglucuronate (**6**)	0.84	0.27	0.32	1.24	0.31	0.25
Hydroxyethyl cellulose (**7**)[f]	0.55	0.25	0.45[g]	–	–	–

[a] H_2O/polysaccharide 2:1, 2 eq NaOH per mole 2-nitroalkyl acetate.

[b] eff = reaction efficiency (ms/ms_{max}), the ms (molar substitution) was calculated by means of the following formula; $ms = 0.01A.N/\{14 - 0.01N(Mwt_{alk} - 1)\}$, A = 162 for 1–2, and 4–5, $A = 153$ for 3, $A = 198$ for 6, and $A = 304$ for 7, N = nitrogen content in %N, Mwt_{alk} = molecular weight of the nitroalkyl substituent. The ms_{max} is the theoretical molar substitution.

[c] The starting materials were contaminated with enzymes and other nitrogen containing material, pullulan %N = 0.28%, guar %N = 0.78%.

[d] No quantitative yields, owing to the low molecular weight and alkaline depolymerization (recovered yields: 2-nitropropyl inulin (42%), 2-nitrobutyl inulin (58%).

[e] H_2O/polysaccharide 1:1.

[f] ms (HE) = 3.23.

[g] H_2O/polysaccharide 4:1.

Table 7
Modified synthesis of 2-nitroalkyl agarose ethers

Conditions	2-nitropropyl acetate		2-nitrobutyl acetate	
	ms_{max}	eff	ms_{max}	eff
2-propanol/H_2O/agarose 26:2:1	0.23	0.24	0.19	0.52
H_2O/agarose 15:1	0.19	0.52	0.26	0.39

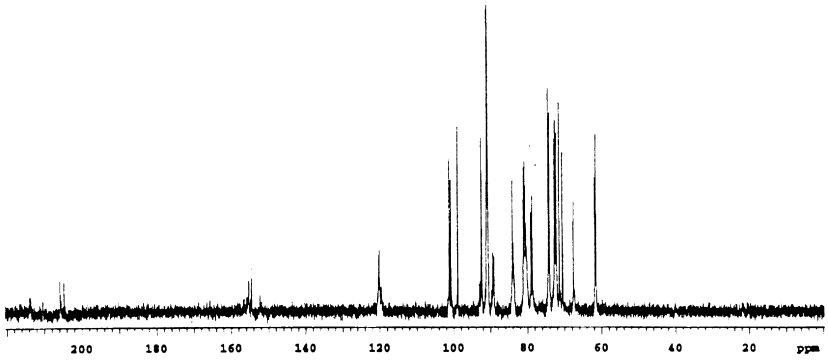


Fig. 1. ^{13}C-NMR of 2-nitropropyl-2-^{13}C-pullulan (50% enriched, $ms_{max} = 0.25$).

hydroxyethylation has a positive effect (eff. = 0.45) on the etherification reaction compared to unsubstituted cellulose, but the efficiency is still rather low in comparison to the other polysaccharides. Most likely etherification occurs both with the hydroxyethyl groups as well as hydroxyl groups of the cellulose backbone.

The absence of a primary alcohol group and/or the negative charge of the uronate are possible explanations for the rather low efficiencies for nitroalkylation of sodium α-polyglucuronate (**6**).

Attempts to modify (nitroalkylation) agarose in aqueous/organic solvents were discouraging (Table 7). Reactions in dilute systems suffer from side reactions, such as hydrolysis of the α-nitroacetoxy-alkanes (Table 7).

3.2. Analysis of 2-nitroalkyl polysaccharide ethers

The incorporation of nitrogen and the appearance of a nitro absorbance in FT-IR spectra (about 1550 cm^{-1}) in all the modified polysaccharides confirm the presence of NO_2 groups in the polysaccharides and indicate a successful Michael addition. Proton NMR spectra of the water-soluble 2-nitroalkyl pullulan, inulin and α-polyglucuronate are rather complex[1] and several multiplets were observed for the methyl protons (δ 1.6–2.2 ppm) of the 2- nitropropyl group) and methylene (δ 0.7–1.1 ppm) and methyl protons (δ 1.8–2.1 ppm) of the 2-nitrobutyl ethers, respectively. Resonances between δ 2.2–3.0 (diastereotopic hydrogens of the methylene group) are consistent with some grafting of nitroalkenes, which occurs when a proton is abstracted from a 2-nitroalkyl ether substituent already present on the polysaccharide. Unfortunately, satisfactory ^{13}C-NMR spectra could not be obtained for any of the abovementioned water-soluble 2-nitroalkyl polysaccharide ethers.

In order to obtain more information about the structure of the polysaccharide derivatives we synthesized labeled 2-nitropropyl-2-^{13}C pullulan (50% enriched, from nitroethane-1-^{13}C, Scheme 3). The ^{13}C-NMR spectrum of this compound is shown in Fig. 1.

New resonances for the labeled methine carbon atom of the starch appear at δ 80–84, δ 89–92, δ 120, δ 154–155 and δ 204–205 ppm. Comparing these results with values obtained from model systems (1-methoxypropane ($CHNO_2$ δ 82 ppm), 1-methoxy-2-methyl-2,4-dinitropentane (CNO_2 2x δ 88–89 ppm (both diastereoisomers)) and an ADS NMR simulation program it can be concluded that the high field resonance (δ 80–84) results from the $CHNO_2$ carbon atom and one of the resonances at about δ 90 ppm originates from the quaternary $C(Me)NO_2$ carbon atom, formed after grafting of 2-nitropropene on a 2-nitroalkyl substituent. The other resonance at about δ 90 ppm most likely signifies the presence of the nitronic acid form (the aci compound, $C{=}NO_2H$) of the 2-nitropropyl functionality. The presence of the nitronic acid tautomer was confirmed by the Konowalow test reaction (Nielsen, 1969). A solution of 2-nitropropyl starch immediately turns red after addition of dilute ferric chloride.

Side products, such as allyl groups (resulting from the elimination of nitrite (Breuer, 1982), allyl pullulan δ

[1] Factors that can explain the complexity of the spectra are: different distributions of the 2-nitroalkyl substituent over the hydroxyl groups of the monomeric unit of the polysaccharide, the introduction of a chiral centre and the nitro functionality can be present as a nitroalkane, nitronic acid (*aci* structure) and sodium nitronate.

Table 8
Reduction of 2-nitropropyl polysaccharides with $Na_2S_2O_4$

2-nitropropyl	$\%N_{st}$	%N (after red.)	$\%NO_2$	%N other	Yield in nitrogen containing material (in %)
Pullulan (**1**a)	2.21	1.82	< 0.05[a]	1.82	82
Guar (**2**a)	2.32	2.00	1.24[b]	0.76	33
Agarose (**3**a)	2.00	1.78	0.45[c]	1.33	67
Inulin (**4**a)	1.40	0.54	nd	nd	nd
α-polyglucuronate (**6**a)	1.71	1.58	–[d]	–	–
HE cellulose (**7**a)	1.09	0.87	0.40[e]	0.47	43

[a] Determined with FT-IR (area 1550/850 cm^{-1} reduced product/area 1550/860 cm^{-1} **1**a).
[b] Area 1550/872 cm^{-1} reduced product/area 1550/872 cm^{-1} **2**a.
[c] Area 1550/932 cm^{-1} reduced product/area 1550/932 cm^{-1} **3**a.
[d] Not detectable due to overlap of the carboxyl functionality.
[e] Area 1550/890 cm^{-1} reduced product/area 1550/890 cm^{-1} **7**a.

120 ppm) and 2-oxopropyl groups (Nef reaction, δ 204–205 (March, 1985)) are incorporated into the polysaccharide to some extent (see Scheme 4 [Products obtained in the synthesis of 2-nitropropylpullulan (the bold values are chemical shifts calculated (ADS program) for the corresponding methoxy compound (Pull = Me), the uncertainty range is given between brackets)]).

The resonance at δ 154–155 ppm originates from sodium nitronate or oximes (*E*, *Z*). A plausible explanation for the formation of oximes is the reaction of 2-nitropropene with the oxygen nucleophile of the nitro/nitronic acid functionality and subsequent decomposition to an oxime and 2-nitro-1-propanal (Breuer, 1982).

No other ^{13}C-labeled polysaccharides were synthesized in this project owing to the high cost of labeled 2-nitropropyl acetate. However, the results obtained for 2-nitropropyl-2-^{13}C pullulan and 2-nitropropyl-2-^{13}C starch[2] suggest the formation of a very similar mixture of products for the other polysaccharides. Unfortunately, the relative amounts of the 2-nitropropyl tautomers and side products could not be determined exactly by integration of the ^{13}C-NMR spectra.

3.3. Reduction of 2-nitroalkyl polysaccharide ethers with $Na_2S_2O_4$ ($NaBH_4$)

As we were interested in a cheap, preferably one-step, synthesis of 2-aminoalkyl polysaccharide ethers, efforts were made to reduce the 2-nitroalkyl ethers with the cheap reducing agent $Na_2S_2O_4$ (Schöter, 1957) and combinations of $Na_2S_2O_4/NaBH_4$ in water. The reductions were performed at room temperature and the pH was maintained at 7 by addition of 1 M NaOH. About 5 equivalents of $Na_2S_2O_4$ (and 2 equivalents of $NaBH_4$) were added to achieve a complete conversion of the nitro group. Sodium dithionite was added in portions to prevent decomposition (Wayman & Lem, 1970).

The products obtained were analyzed with elemental analysis (nitrogen content) and FT-IR. As the absorbance of the nitro functionality at 1550 cm^{-1} increases (or decreases) linearly with the molar substitution of the modified polysaccharide the yield of new N-containing polysaccharide ethers can be determined by combining elemental analysis with IR spectroscopy. The yield (Table 8, fifth column) can be estimated by subtracting the %N present in the form of the nitro groups (Table 8, third column) from the total nitrogen content of the product isolated (Table 8, second column) and dividing this by the nitrogen content of the starting material.[3] The results of the reduction of the 2-nitropropyl and 2-nitrobutyl derivatives of **1**–**7** are given in Table 8–10.

The yield in nitrogen containing material after the reduction of 2-nitropropyl and 2-nitrobutyl polysaccharides (determined by FT-IR spectroscopy and elemental analysis) is moderate to high. The low efficiency for the reduction of 2-nitropropyl guar (**2a**) is probably an artifact. The effect of simultaneous addition of $NaBH_4$ is rather small and does not improve the conversion of the nitro functionality significantly.

3.4. Analysis of reduced 2-nitroalkyl polysaccharides

The almost complete conversion of the nitro functionality for the reduction of the 2-nitroalkyl polysaccharides described in Tables 8–10 unfortunately does not necessarily imply that the corresponding aminoalkyl polysaccharides are formed. Intermediates in the reaction (e.g. nitroso, oxime, hydroxylamine) may be stabilized during the reaction or side reactions may occur. Owing to the low degrees of substitution no direct information about the product formation can be obtained with FT-IR. Nitroso, oxime,

[2] Heeres, A., van Doren, H.A., Gotlieb, K.F., Bleeker, I.P., Kellogg, R.M. Submitted for publication in Carbohydrate Research.

[3] The decrease in molar weight is rather slight, owing to the low molar substitution.

Table 9
Reductions of 2-nitrobutyl polysaccharides with $Na_2S_2O_4$

2-nitrobutyl	$\%N_{st}$	%N (after red.)	$\%NO_2$	%N other	Yield in nitrogen containing material (in %)
Pullulan (**1**b)	1.71	1.54	< 0.05[a]	1.54	90
Guar (**2**b)	2.22	1.77	0.16[b]	1.61	73
Agarose (**3**b)	1.47	1.59	0.74[c]	0.85	58
Inulin (**4**b)	2.22	0.38	nd	nd	nd
α-polyglucuronate (**6**b)	1.32	1.10	–[d]	–	–

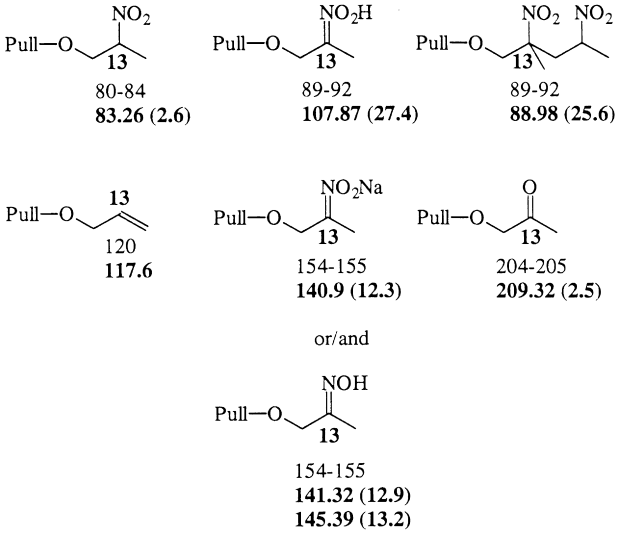
[a] Determined with FT-IR (area 1550/850 cm^{-1} reduced product/area 1550/860 cm^{-1} **1**b.
[b] Area 1550/872 cm^{-1} reduced product/area 1550/872 cm^{-1} **2**b.
[c] Area 1550/932 cm^{-1} reduced product/area 1550/932 cm^{-1} **3**b.
[d] not detectible due to overlap of the carboxyl functionality.

Table 10
Reductions of 2-nitropropyl polysaccharides with $Na_2S_2O_4/NaBH_4$

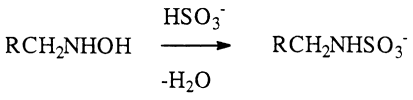
2-nitropropyl	$\%N_{st}$	%N (after red.)	$\%NO_2$	%N other	Yield in nitrogen containing material (in %)
Pullulan (**1**a)	2.21	1.75	0.31	1.44[a]	65
Guar (**2**a)	2.32	2.47	0.19	2.28[b]	98
Agarose (**3**a)	2.00	1.66	< 0.05	1.66[c]	83
HE cellulose (**7**a)	1.09	0.76	< 0.05	0.76[d]	70

[a] Determined with FT-IR (area 1550/850 cm^{-1} reduced product/area 1550/860 cm^{-1} **1**a.
[b] Area 1550/872 cm^{-1}/area 1550/872 cm^{-1} **2**a.
[c] Area 1550/932 cm^{-1}/area 1550/932 cm^{-1} **3**a.
[d] Area 1550/890 cm^{-1}/area 1550/890 cm^{-1} **7**a.

hydroxylamines and amines give rise to rather low or medium absorbances in the 1500–1700 cm^{-1} region (Heeres et al., 1997) and these absorbances cannot be observed clearly owing to strong polysaccharide/water absorbances at these wavelengths.



Scheme 4.



Scheme 5.

Negative ninhydrin tests for **1**a–**6**a exclude significant formation of amino functionalities.[4] In order to get a better insight into the reduction process 2-nitropropyl-2-^{13}C-pullulan (50% enriched) was reduced with $Na_2S_2O_4$ and $Na_2S_2O_4/NaBH_4$. In the ^{1}H-NMR spectra the resonance of the methyl group of the 2-nitropropyl ether (δ 1.6–2.2 ppm) shifts to higher field (δ 1.2–1.8 ppm). The ^{13}C-NMR of reduced 2-nitropropyl-2-^{13}C-pullulan ($Na_2S_2O_4/NaBH_4$) is shown in Fig. 2.

Compared to the starting material (Fig. 1), new resonances appear at δ 48–54 ppm and δ 60–68 ppm. The resonances at δ 120 (allyl), δ 155–157 (sodium nitronate/oxime) and 204–205 ppm (carbonyl) have disappeared and the intensities of the resonances at δ 80–82 (nitroalkane) and δ 90–92 (nitronic acid/grafting) have become smaller. Resonances of the carbonyl and oxime remain

[4] A ninhydrin test of the amino polysaccharide chitosan does give the characteristic blue coloring.

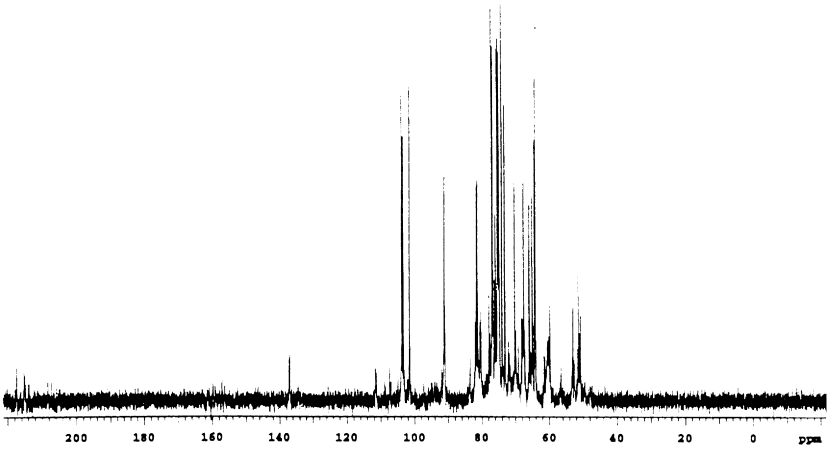


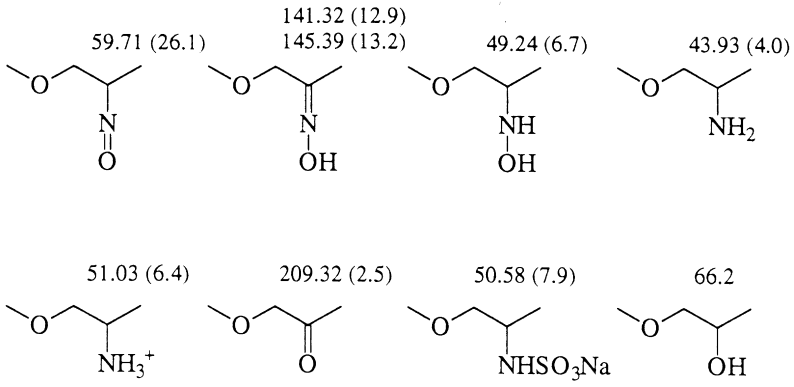
Fig. 2. ^{13}C-NMR spectrum of reduced 2-nitropropyl-2-^{13}C-pullulan ($Na_2S_2O_4/NaBH_4$).

present in the reduced product when no $NaBH_4$ is added during the reduction.

NMR-tube reactions of the model systems 1-methoxy-2-nitropropane and 1-methoxy-2-nitrobutane with $Na_2S_2O_4$ proceeded rather selectively (80–90%) and a major product was formed in which new resonances for the methine carbon appeared at δ 48.51 and δ 54.13 ppm. The chemical shifts are in reasonable agreement with the chemical shift for the methine carbon of 1-methoxy-2-aminoalkanes, the corresponding hydroxylamines or the sulfamates (Scheme 5 [Formation of sulfamates from hydroxylamines]). After preparative reactions, the compounds could not be isolated from the water layer by extraction with organic solvents and, therefore, we could not determine their exact nature. However, because of the very good water solubility of the product formed, it seems likely that sulfamates are formed under these reaction conditions (Na_2CO_3 is used to maintain a constant pH). It has been reported in the literature that reduction of nitro and nitroso-compounds with $Na_2S_2O_4$ may indeed lead to formation of sulfamates if the reduction is performed under neutral or slightly alkaline conditions (Glaser, Möller, Pieper, Schröter, Spielberger & Söll, 1957; Pecorari, Rinaldi, Costi & Antolini, 1992). Their formation is explained by reaction of the hydroxylamine intermediate with bisulfite (HSO_3^-, Scheme 6 [Calculated ^{13}C-NMR values of several reaction products of the methine group of reduced 1-methoxy-2-nitropropane (the calculated uncertainty range is given between brackets)]). Bisulfite is formed in large quantities during the decomposition of $Na_2S_2O_4$ and in the reduction step as well.

Based on these results and determination of chemical shifts (ADS, see Scheme 6) the new resonances most likely originate from nitroso compounds, hydroxylamines, hydroxypropyl substituents and sulfamates.

The formation of sulfamates is confirmed by sulfur analysis of reduced sodium α-polyglucuronate (%S = 0,84%, %N = 1.58%, yield in sulfamate = 21%). It can be concluded that the reduction of 2-nitroalkyl polysaccharides with $Na_2S_2O_4$ or $Na_2S_2O_4/NaBH_4$ does not go to completion and several intermediates (probably stabilized during the reaction) are formed. It is likely that a complete reduction with excess $Na_2S_2O_4$ initially leads to the formation of a sulfamate, formed by the reaction of the hydroxylamine with bisulfite.



Scheme 6.

4. Conclusions

2-Nitroalkyl polysaccharide ethers can be synthesized by Michael additions of polysaccharides to 2-nitroalkenes, formed in situ from β-nitro-acetoxy-alkanes. The efficiency of the reaction depends on the accessibility of the polysaccharide for the substrate. High efficiencies (60-95%) are obtained for pullulan, guar, agarose, and inulin when the Michael additions are performed under alkaline conditions in highly concentrated (homogeneous or heterogeneous) aqueous systems.

As judged from the ^{13}C-NMR of ^{13}C-enriched 2-nitropropyl pullulan the nitro functionality exists as a mixture of the nitroalkane, nitronic acid and sodium nitronate. Grafting occurs and products of side reactions, e.g., the Nef reaction (carbonyl), and the splitting off of nitrite (allyl) are incorporated into the polysaccharide.

Reduction of 2-nitroalkyl polysaccharides with $Na_2S_2O_4$ (5 equivalents) or a mixture of $Na_2S_2O_4/NaBH_4$ leads to complex polysaccharide ethers which are most likely mixtures of nitro and nitroso compounds, hydroxylamines, hydroxypropyl ethers and sulfamates. Further research is needed to achieve a selective conversion of the nitro functionality to aminoalkyl polysaccharides.

Acknowledgements

We sincerely thank AVEBE BA. for enabling us to buy nitroethane-1-^{13}C and supporting our research project.

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